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# Experimental Study of the Influence of Fluorine Administration on Tuberculous Lesions in Rabbits

First Report: Method of determining the fluorine  
content of animal tissue

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## I. Introduction

As a preliminary experiment we investigated the methods of determining the fluorine content of animal tissues.

Since the report by Willard and Winter<sup>(1)</sup> on the isolation and the quantitative determination of fluoride in tissues many attempts to improve it have been made by investigators. Recently Huckabey, Welch and Metler<sup>(2)</sup> reported a new method, which simplifies the control of the distilling temperature by use of a bath containing tetrachlorethane (B.P. 146°C). By this method the steam distillation of fluorine becomes very easy.

Since De Boer<sup>(3)</sup> proved that the colorimetry with zirconium-alizarin reagent was useful in the determination of fluoride in aqueous solution, several improvements have been made. Taras<sup>(4)</sup>, Megregian and Maier<sup>(5)</sup> improved this method by using a photoelectric colorimeter.

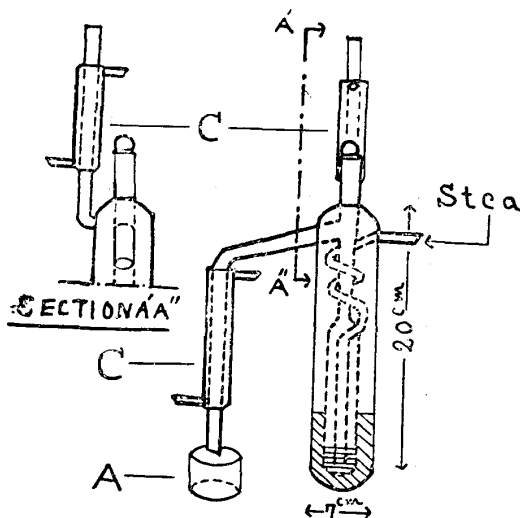
Therefore, we have performed the following experiments by the methods described by these authors.



## II. Method and procedure

Add 1-2 ml. of 10% magnesium acetate solution and 0.1 ml. of N/30 NaOH solution to a dried sample of animal tissue (0.1-3.0 g) and dry again. Ignite it in an electric furnace at 500-600°C until ashing is complete as indicated by a white or gray ash. After cooling, crush the ash to a powder with a pestle, mix

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Figure



|   |   |
|---|---|
| A   | Beaker                                  |
| C   | Condenser                               |
|  | Tetrachlorethane                        |
|  | Sample + H <sub>2</sub> SO <sub>4</sub> |

thoroughly, and weigh. Record weight of total ash and store in a stoppered bottle. Transfer this ash with 5 ml. of water and 10 ml. of sulfuric acid to Huckabey's apparatus, and heat over a burner. After boiling of tetrachlorethane starts, steam distillation is begun. Receive the distillate in a beaker containing 5 ml. of 0.03 N NaOH solution. Add 5 ml. of zirconium and alizarin reagents for each 100 ml. of distillate, and place upright at room temperature for about an hour and then determine the fluorine content by a photoelectric colorimeter at 535  $\mu$ m.

### III. Results

(1) The relationship between the recovered quantity of fluorine and the quantity of distillate is presented in Table

1. 50–100  $\mu$ g. of fluorine can be recovered almost completely in 200–300 ml. distillate. Huckabey reported the recovering of about 98 % of added fluorine in 50 ml and about 100 % in 100 ml. of distillate. Our experiments are a little slower in the distillation than Huckabey's.

Table 1

| No.                      | F added ( $\mu$ g) | Volume of distillation (ml) | F recovered, % |
|--------------------------|--------------------|-----------------------------|----------------|
| 1                        | 50                 | 100                         | 93             |
| 2                        | 50                 | 200                         | 96             |
| 3                        | 100                | 100                         | 92             |
| 4                        | 100                | 200                         | 100            |
| 5                        | 100                | 300                         | 98             |
| 6                        | *100               | 200                         | 96             |
| *HCl <sub>4</sub> : used |                    |                             |                |

#### (2) Interfering ions:

The known interfering ions in the colorimetry by the zirconium-alizarin method are Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Al<sup>3+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup> and PO<sub>4</sub><sup>3-</sup>. The transfer of these ions in distilled water into distillates during the distillation is shown in Table 2.

Table 2

| ION \ No.                      | 1   | 2   | 3    | 4    | 5    | 6   | Added   |
|--------------------------------|-----|-----|------|------|------|-----|---|
| SO <sub>4</sub> <sup>---</sup> | (-) | (-) | (-)  | 3    | (-)  | (-) | H <sub>2</sub> SO <sub>4</sub> 5 ml                             |
| Al <sup>+++</sup>              | (-) | (-) | (-)  | 0.07 |      |     | KAl(SO <sub>4</sub> ) <sub>2</sub> • 12H <sub>2</sub> O 4.74 mg |
| Mg <sup>++</sup>               | (-) | (-) | (-)  | (-)  |      |     | MgSO <sub>4</sub> • 7H <sub>2</sub> O 49.2 mg                   |
| Fe <sup>+++</sup>              | (-) | (-) | 0.01 | 0.03 | (-)  | (-) | FeCl <sub>3</sub> • 6H <sub>2</sub> O 16.2 mg                   |
| PO <sub>4</sub> <sup>---</sup> | (-) | (-) | 0.01 | 0.3  | 0.03 | (-) | Na <sub>2</sub> HPO <sub>4</sub> • 12H <sub>2</sub> O 53.7 mg   |
| Unit: PPM                      |     |     |      |      |      |     |   |

The amounts of these ions transferred into the distillate were much less than the concentrations which had been shown by Megregian and Maier to interfere in zirconium-alizarin colorimetry. Concerning Cl<sup>-</sup> and PO<sub>4</sub><sup>---</sup> ions the following experiments were performed.

A sample 2.5 g. of dried bovine liver powder was used, and the concentrations of Cl<sup>-</sup> and PO<sub>4</sub><sup>---</sup> ions in the distillates were determined. The results are presented in Table 3.

The concentrations of both Cl<sup>-</sup> and PO<sub>4</sub><sup>---</sup> ions were much lower than the known interfering values (Cl<sup>-</sup>: 100~300 ppm PO<sub>4</sub><sup>---</sup>: 1~5 ppm). Therefore, in the cases of small amounts of tissue samples

Table 3

| ION \ No.                      | 1    | 2    | 3    | 4     | 5     |
|--------------------------------|------|------|------|-------|-------|
| Cl <sup>-</sup>                | 1.25 | 1.03 | 2.85 | 0.88> | 0.67> |
| PO <sub>4</sub> <sup>---</sup> | 0.01 | 0.06 | 0.04 | 0.01  | 0.06  |

Cl<sup>-</sup> and PO<sub>4</sub><sup>---</sup> ions transferred in the distillates have no hazardous effects upon the determination of fluorine by colorimetry with zirconium-alizarin reagents.

(3) Preliminary experiments of the quantitative determination of fluoride in the bones of rabbits to which fluorides (NaF) have been fed:

Rabbits were fed 40 mg NaF daily for about 7 months. 0.1 g of dried bone powder of these animals was treated by the method described above and their fluorine contents were determined. Then 10 ml. of these distillates was double distilled again after adding 10 ml. of HClO<sub>4</sub>. The values of fluorine content obtained from single and double distillations are described in Table 4.

Almost no differences are seen between the values of the two treatments. Therefore, in the case of small amounts of tissue samples double distillation is not necessary.

Table 4

| No. | Distillation (μg) |        |
|-----|-------------------|--------|
|     | Single            | Double |
| 1   | 92                | 91     |
| 2   | 94                | 93     |
| 3   | 90                | 78     |
| 4   | 89                | 89     |
| 5   | 91                | 90     |
| 6   | 86                | 86     |
| 7   | 90                | 86     |
| 8   | 94                | 89     |
| 9   | 93                | 91     |
| 10  | 91                | 87     |

### III. Summary

For the purpose of the quantitative determination of fluoride in tissue specimens we examined the distillation method of Hackabey et al and the colorimetric determination by the Zirconium-alizarin method. With Huckabey's apparatus it is necessary to obtain more than 200 ml. of distillate in order to recover the fluorine completely.

In the case of small amounts of tissue samples (under 5 g) the isolation and the determination of fluoride can be performed without regard to the influences of interfering ions, and so-called double distillation is not necessary.

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